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HISTOLOGICAL DISTRIBUTION AND RETENTION OF LEAD, RADIOACTIVE SULFUR, AND RADIOACTIVE CALCIUM IN COMPACT BONE

J. Vincent

ABSTRACT: The pattern of deposition of new bone substance in dogs and monkeys is investigated by histological means with lead and by radiological means with X-rays and radioactive isotopes, including calcium-45. It is found that the new deposit is at first calcium-poor and then undergoes massive calcification, during which a radioactive isotope can be co-deposited. Some inferences with regard to uptake and both short-term and long-term retention of radioactive isotopes by the animal or human skeleton are drawn, and avenues for further research are mentioned.

Morphological research on the constitution of bone substance has benefited greatly from little-known or only sketchily known techniques. Among those methods, X-ray photomicrography ranges among the most fruitful. Its application to compact bone dates from barely a few years ago (Amprino, 1952; Amprino and Engström, 1952). A section of diaphysis reduced by attrition to a thickness of about 50 microns is placed on a very fine-grain emulsion. The X-ray picture is obtained by the use of very soft X-rays and is placed under the microscope as anordinary histological preparation. X-ray photomicrography offers an appreciable advantage: after it has yielded the pattern of calcification of a section, it leaves the section intact for any further investigation. /41*

For example, let us contrast the X-ray photomicrograph and the histological image of the same region of a section of compact bone, (Lacroix, 1954; Vincent, 1954 and 1955). The comparison demonstrates that an osteon develops in three phases, proceeding from a resorption cavity. The walls of this cavity are at first lined with layers that are visible on the section, but are not detected by the X-rays and hence contain hardly any calcium. These lamellae of very recent formation have been called central margins and, later, preosseous margins. This tissue suddenly undergoes a massive calcification: it then has about two-thirds the charge of calcium that it will posses when it is saturated (Amprino, 1952). The last phase of maturation of the osteon comprises rapid completion of its mineralization.

In the course of our presentation, the X-ray photomicrograph

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* Numbers in the margin indicate pagination in the foreign text.

will serve as our reference image, to which we shall refer the distribution of the lead detected by histochemistry and the distribution of radioactive sulfur or radioactive calcium indicated by tracer photography.

SHORT-TERM DISTRIBUTION OF RADIOACTIVE CALCIUM AND OF LEAD

Tracer photography with radioactive calcium will give us a better view of the dynamics of calcification of compact bone than would X-ray photomicrography. The mode of distribution of radioactive calcium administered to the dog has already been illustrated (Lacroix, 1953, 1954, and 1956; Vincent, 1955). The results are identical in the case of *Cercopithecus*. If the animal is killed during the first week after the injection, the emulsion records a weak background reaction and, most especially, very marked imprints of radioactivity /42 corresponding to osteons in process of deposit. More precisely, the radioactive calcium is deposited in the most central calcified stratifications of these osteons. If the X-ray photomicrograph and tracer photograph are superposed, it is found that the radioactivity pertains to the periphery of the haversian canal as it is imaged on the X-ray photomicrograph.

Other radioactive elements are similar in behavior to calcium-45. They are phosporus-32 (Engfeldt, Engström and Zetterström, 1952), strontium-90 (Jowsey, Owen and Vaughan, 1953; Engfeldt, Björnerstedt, Clemedson and Engström, 1954), and gallium-72 (Dudley, Iminie and Istock, 1950).

It seemed of interest to know whether lead introduced into the organism distributes itself in accordance with the same pattern in compact bone (Vincent, 1957). This time, tracer photography gave way to histochemistry. For a week, dogs received, as an admixture to their food, a solution of 4% basic lead acetate in a dosage of 50 mg of acetate per day per kilogram of animal. The lead in the bone sections is detected with sodium sulfide.

The distribution of the lead, like that of calcium-45, is not uniform. Only certain osteons took up an appreciable quantity of lead. When they are examined more closely, it is observed, the day after the experiment, that the dyed layers coincide with the innermost layers visible by X-ray in the forming osteons. In other words, the lead shows up at the precise place where the matrix is suddenly and massively calcified.

Our own experiments and the statements in the literature imply that certain elements can, within a short term, be deposited electively in the zones of rapid calcification.

SHORT-TERM DISTRIBUTION OF RADIOACTIVE SULFUR

We have just seen that several possibilities are available to the morphologist who wishes to study the mineral metabolism of bone substance. On the other hand, mineralization is preceded by the development of the organic portion. How can this development be traced? We inject sulfur-35 into a monkey and investigate it four days later on a tracer photograph. This time, the radioactive traces show up within the calcified lamellae of the forming osteons; the radioactive sulfur has been deposited in the preosseous margin. This proposition has already been established in the dog (Lacroix, 1954, and 1956; Vincent, 1954 and 1955).

For spongy bone, the pictures are analogous: the radioactivity borders on the X-ray shadow of the trabecula but does not overlap it. Let us note in passing that the spongy bone substance of the adult does not essentially differ from the compact substance (Vincent, 1955; Lacroix, 1956). However, we shall prefer to concentrate on the diaphysis, because the study of the epiphyses is impeded by a great many technical difficulties and provides pictures whose interpretation is rather complicated.

CONSTITUTION OF THE MATRIX /43

Tracer photography with sulfur has been considered as a method of vital coloration of sulfomucopolysaccharides (Boström and Odeblad, 1953). Its indications deserve in any case to be rounded out by histology and, in particular, by the methods that are reputed to indicate the presence of mucopolysaccharides.

Once they have been photomicrographed by X-ray, the sections of monkey bone are decalcified in sequestrene before being dyed.

The McManus test produces a positive reaction to P.A.S. in the preosseous margin. The other regions of the section are tinted very slightly pink.

Methylene blue at varying pH shows that the basophilism of the preosseous margin vanishes below pH 4, that the basophilism of the zones in process of calcification vanishes below pH 4.8, and that the rest of the matrix is not dyed below pH 5.6.

After dyeing with toluidine blue, the preosseous margin appears orthochromatic, and the rest of the forming osteons, metachromic.

Except for a few details, these results are in agreement with the observations we have gathered in man and in the dog.

Thus, it is evident that the matrix is modified suddenly at the moment it begins to calcify. Immediately below, we shall see that radioactive sulfur remains incorporated in the matrix in the course of its transformations. Biochemistry, moreover, recently has particularized the place of sulfur in the bone substance: after administration of sulfur-35 to the rabbit, the radioactive material, extracted from the decalcified bone, is simply chondroitin sulfate or a closely related substance (Kent, Jowsey, Steddon, Oliver and Vaughan, 1956).

CHRONOLOGY OF THE HAVERSIAN TRANSFORMATION

Hence, we now have a means of labeling at will the organic portion or the inorganic portion of the bone at the moment when it is deposited. This resource was exploited to evaluate the rate of the figured transformations of compact bone. Such an investigation was performed in the adult dog with radioactive sulfur (Vincent, 1955; Lacroix, 1956). However, as lead offers advantages for a study of this kind, we employed it in the same species to confirm the information supplied by radioactive sulfur (Vincent, 1957).

If lead is administered to an animal during two weeks separated by a one-week interval, two concentric dyed rings will be found in certain osteons. The dimensions of these rings and the undyed band intervening between them permit an estimate of about 10 microns for the thickness of the bone layer that forms in an osteon in a period of one week.

If, next, the animal is killed six weeks after the administration of the lead, the dyed zone is detected at the periphery of the osteon, which during that period of time has progressively built up /44
a resorption cavity. Hence, the phase of deposit of an osteon requires about six weeks.

After three months and a half, the rings of lead are still visible. At this stage, however, the osteons have not yet completed their calcification. Therefore, this last phase of development of osteons is much slower than the preceding ones.

Before commenting on the long-term experiments, let us add an important remark. In certain animals that exhibit no differences on first view, the haversian transformations seem to indicate a time lag. Indeed, an injection of calcium-45 in a dog (Vincent, 1955) and an injection of sulfur-35 in a monkey gave us merely a diffuse reaction on the tracer photographs: no imprints of high radioactivity are found in the compact bone. Furthermore, the histological examination does not reveal any preosseous margin.

It is appropriate to emphasize that the abundance of labeled osteons differs perceptibly from one animal to another. The degree of affinity of the skeleton for many elements depends on as yet poorly defined factors, among which the age of the subject no doubt plays a leading part.

LONG-TERM EXPERIMENTS

Besides lead, which has already been mentioned, both radioactive calcium and radioactive sulfur have been detected after four months in the adult dog, in intact osteons (Vincent, 1955; Lacrois, 1956). Prolonged retention of strontium-90 has been found by tracer photography in the rabbit (Jowsey, Owen, Tutt and Vaughan, 1955);

of carbon-14, in the mouse (Skipper, Nolan and Simpson, 1951); and of radium, in man (Hoecker and Roofe, 1951; Looney and Woodruff, 1953; Looney, 1956). The documents concerning radium are the more interesting since they derive from man and the time elapsed between exposure and death of the subject is, in one case, as much as 25 years. The documents suggest that in the adult the replacement of old bone by new is very slow.

Radioactive sulfur also seems to be incorporated very stably. One of our monkeys, killed six months after the injection, yielded tracer photographs on which the radioactivity corresponds to completely calcified intact osteons.

These observations show that an element taken up by the bone substance, either by its organic fraction or by its inorganic fraction, can remain there for a very long time without migrating markedly. In this regard, the skeleton constitutes a notable exception among the tissues of the organism.

GENERAL DISCUSSION

In association with X-ray photomicrography and histology, the tracer photograph technique has proved to be a convenient instrument for the study of the figured transformations of bone substance. Moreover, lead seems capable of rendering excellent service in this field. It is, furthermore, of very particular interest because of /45 its own toxicological importance and its place in the families of natural radioactive isotopes.

Hence, it is undeniable that a better knowledge of the bone substance can, by itself, orient research designed to combat the uptake and retention of noxious elements in the skeleton. Let us repeat that the starting point for our work is morphological and that we have analyzed only the histological transformation of the skeleton. The metabolic transformation and the ion exchanges are not covered.

Be that as it may, our observations permit us to establish certain facts that can settle the problems of protection associated with the development of the nuclear sciences.

Not all skeletons retain the same quantity of a given element. The factors that determine these variations have yet to be studied.

Many radioactive isotopes are deposited in the zones of osteogenesis or on their immediate peripheries. Thus, they are concentrated in small spaces, rather than being uniformly distributed, for which reason they are all the more dangerous.

Lastly, the very slow pace of the figured transformations of the skeleton can prolong almost indefinitely the retention of undesirable substances.

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